GEOPHYSICAL RESEARCH LETTERS, VOL. 36, L01301, doi:10.1029/2008GL036436, 2009

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CO_2 evolution and short-term carbon turnover in stable soil organic carbon from soils applied with fresh organic matter

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Received 24 October 2008; accepted 13 November 2008; published 1 January 2009.

[1] The organic carbon of particle size <53 μm is mineral-associated organic carbon (MAOC), the measurable fraction of passive soil organic matter pool described in CENTURY model. We studied the effect of fresh organic matters (FOMs): no OM (control); chicken manure (CM): 2.12 g CM carbon kg^{-1}; and leaf litter (LL): 1.81 g LL carbon kg^{-1} on short-term dynamics of MAOC and CO_2 evolution of two soils: Bagabag, Philippines (121°15′E, 16°35′N) and Tsumagoi, Japan (138°30′E, 36°30′N). Cumulative CO_2 evolution was significantly higher in CM-applied soils. Significant MAOC decrease in 5–20-cm depth of Tsumagoi soil suggest short-term stable C turnover even with FOM application. Greater MAOC decline in CM-applied Bagabag soil suggest that manure application may result to bigger stable C turnover in this soil. Our results provide evidence of significant short-term stable SOC turnover, and challenge the convention that only labile SOC is involved in short-term CO_2 evolution from soils. **Citation:** Dumale, W. A., Jr., T. Miyazaki, T. Nishimura, and K. Seki (2009), CO_2 evolution and short-term carbon turnover in stable soil organic carbon from soils applied with fresh organic matter, *Geophys. Res. Lett.*, *36*, L01301, doi:10.1029/2008GL036436.

1. Introduction

[2] In recent years, a variety of terrestrial ecosystem models have been developed to study the impacts of management and/or climate change on soil organic carbon (SOC) turnover under different climates, topographies and management [*Sherrod et al.*, 2005]. The CENTURY model is a terrestrial SOC model which partitions SOC into three conceptual pools: active, slow, and passive, which differ in turnover times [*Parton et al.*, 1988]. From the literature, we summarized the relationships of the measurable fractions of these conceptual pools and their measurable fractions with the particle size fractions (Table 1). The mineral-associated organic carbon (MAOC) is the measurable fraction of the passive SOC pool [*Sherrod et al.*, 2005]. The MAOC fraction can be measured by physically separating the <53 μm particle size fraction, which is the silt-and clay-sized fraction [*Haile-Mariam et al.*, 2008]. The associated SOC of the combined silt and clay is the MAOC [*Cambardella and Elliot*, 1992].

[3] Most of the SOC in soil (60–70%) resides in the passive pool [*Parton et al.*, 1988] and little turnover from this pool can significantly affect the overall terrestrial C dynamics. The stable pool is structurally and chemically protected in the fine fractions of the soil, and is conventionally believed as not a source of extra carbon turnover, especially in the short-term. In areas that receive natural or artificial fresh organic matters (FOM), the scenario of a MAOC-contributed CO_2 and C turnover may bring into doubt the extent of the capacity of soils to store carbon. This may constitute a previously unrecognized source of additional carbon turnover, considering the abundant use of FOM in agricultural ecosystems and natural occurrence of forest litter in the highlands.

[4] Findings have been contradictory over if organic matter (OM) application increases SOC [*Kuzyakov et al.*, 2000]. Some studies suggested OM application does not increase SOC [*Bell et al.*, 2003; *Campbell et al.*, 1991; *Foereid et al.*, 2004; *Fontaine et al.*, 2003, 2004], while others have reported gains in SOC after years of OM addition to soil [*Gerzabek et al.*, 1997, 2001; *Dalenberg and Jager*, 1989]. It seems unlikely that only the labile SOC pool is susceptible [*Hamer and Marschner*, 2005] and that the additionally released CO_2 can originate from the different pools of SOC [*Kuzyakov and Bol*, 2006]. Findings of labeling studies provided indirect evidence of stable SOC pool-contributed primed carbon during microbial respiration [*Bell et al.*, 2003; *Luna-Guido et al.*, 2001; *Fontaine et al.*, 2004; *Vanlauwe et al.*, 1994]. In our experiment, we separated the MAOC fraction of two Asian soils by aid of chemical dispersion and physical fractionation [*Haile-Mariam et al.*, 2008; *Sherrod et al.*, 2005; *Cambardella and Elliot*, 1992] to directly measure the short-term dynamics of stable SOC as affected by FOMs application. We hypothesized that although the MAOC is physically protected in the silt and clay fractions, it does contribute to C turnover in the short-term, although conventionally believed to be stable and turn over in centuries to millennial timescales.

2. Incubation Experiment

[5] Transparent 500-mL glass bottles were used for incubation. It was mounted on the lid with an acrylic tube fitted with a rubber septum which served as gas sampling port. In addition, two 3-way acrylic valves mounted on the lid served as outlets for the air flushed from the bottles and inlet for fresh air to replenish the O_2 inside the bottles every sampling day. The experimental units consisted of 20-g soil adjusted to 50% of the soil's water-holding capacity. Incubation was conducted for 110 days at 20°C constant temperature. Prior to airtight sealing of the bottles, the chicken manure (CM) and leaf litter (LL) were evenly mixed with the soil at a computed rate of 2.12 g chicken manure C kg^{-1} soil; 1.81 g leaf litter C kg^{-1} soil. We used

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0094-8276/09/2008GL036436$05.00

Table 1. Matrix Table Indicating Relationships of Conceptual SOM Pools, Their Measurable Fractions, and Particle Size Fractions

	Conceptual SOM Pools[a]		
Description	Active	Slow	Passive
1. Turn-over time	hours to months;[b,c] 2- to 4-years[d]	decadal;[b] 20- to 50-years[d]	centuries to millennia;[b] 800–2000 years[d]
2. Representative SOM Fraction[e]	SMBC (soil microbial biomass carbon)[b,f,g,h]	POMC (particulate organic matter carbon)[b,i]	MAOC (mineral-associated organic carbon)[b,i]
3. Description of the fraction[d]	active soil organic matter (SOM) consisting of live microbes and microbial products	protected fraction that is more resistant to decomposition	physically-protected or chemically resistant and has long turnover time
4. Chemical composition[j]	chloroform-labile, microwave-irradiation-labile SOM, amino compounds, phospholipids	amino compounds; glycoproteins; aggregate protected POM; acid/base hydrolyzable; mobile humic acids	aliphatic macromolecules; charcoal; sporopolleins; lignins; high molecular, condensed SOM, humin, nonhydrolyzable SOM, fine silt, coarse-clay associated SOM
5. SOM fraction association with soil particle sizes	Fumigated and extracted SMBC[b,k]	2 mm – 53 μm;[b,i,l] sand-sized or larger[j]	<53 μm,[b,i,l] silt and clay-sized[b,l,m] referred to as MAOC in this paper

[a]The term "pool" is used to refer to the theoretically separated, kinetically delineated components of SOM.
[b]*Sherrod et al.* [2005].
[c]*Follett* [2001].
[d]*Parton et al.* [1988].
[e]The term "fraction" is used to describe measurable organic matter components associated with the pool.
[f]*Davidson et al.* [1987].
[g]*Franzluebbers et al.* [2000].
[h]*Franzluebbers et al.* [1996].
[i]*Cambardella and Elliot* [1992].
[j]*Wander* [2004].
[k]*Vance et al.* [1987].
[l]*Haile-Mariam et al.* [2008].
[m]Silt and clay-sized particles are <50 μm diameter based on the USDA Soil Texture Classification System.

commercially-available processed chicken manure and leaf litter purchased in Tokyo, Japan. They were finely ground, passed through a 0.5-mm mesh screen, and stored at 4°C before the experiment. Leaf litter had air-dried moisture content of 15.6% and chicken manure 14.2% upon incorporation. We analyzed total C and N using a Sumigraph NC-90A NC analyzer (Sumika Inc.) prior to soil incorporation (chicken manure: 424.9 g kg^{-1} C; 52.5 g kg^{-1} N; 8.1 C/N ratio; leaf litter: 362.7 g kg^{-1} C; 18.0 g kg^{-1} N; 20.1 C/N ratio). Sufficient number of experimental units was prepared to allow for three replicates per treatment for each sampling day.

3. Separation and Measurement of the MAOC Fraction

[6] We used a combined chemical dispersion and particle size separation method based on the work of several authors [*Haile-Mariam et al.*, 2008; *Sherrod et al.*, 2005; *Cambardella and Elliot*, 1992] to separate the combined silt- and clay-sized fractions which contain the MAOC. The MAOC was determined by destructive sampling at 0, 3, 13, 21, 44, 70, 85, and 110 days after incubation. On each sampling day, we first took headspace gas samples for CO_2 evolution measurement before taking a 5-g subsample for MAOC measurement. For <53 μm size separation, the 5-g subsample was dispersed with 50 mL of sodium hexametaphosphate (5 g/L) in a 100-mL plastic bottle and shaken in a reciprocating shaker overnight at 240 rpm. The soil suspensions were sieved with a 53-μm screen (Tokyo Screen Co. Ltd., Japan), and all particles passing through the screen were dried overnight at 70° C. The dried samples were finely ground manually using mortar and pestle to pass through an 80-μm sieve, and MAOC was measured using a Sumigraph NC-90A NC analyzer (Sumika Inc.). We performed statistical analysis using IRRIStat for Windows (version 5) to determine any significant relationship between and among treatments.

4. Gas Sampling and CO_2 Evolution Measurement

[7] Gas samples for CO_2 evolution measurement were drawn using a 10-mL plastic syringe (Nipro, Japan) fitted with 0.70 × 38.00 mm needle (Nipro, Japan). Before each sampling date, transparent 7-mL capacity vacuum glass vials were prepared by subjecting to 2 millibars suction for about 10 min and carefully sealed with a rubber septum. Prior to drawing the gas sample, the air inside each incubation bottle was homogenized by continuous pumping and sucking using the sampling syringe for about 4–5 times before gas samples were taken. Approximately 7 mL of gas samples were injected into the vacuumed vials where 1 mL of gas sample was drawn and injected into a 16A Gas Chromatograph (Shimadzu Inc.).

5. Results

5.1. CO_2 Evolution

[8] CO_2 evolution rate was in the order CM > LL > control in both soil (Figure 1). Peak CO_2 evolution rate in the Bagabag soil occurred within the first 3 days of incubation. The CM-applied soils had peak CO_2 evolution rates of 166.52 and 174.92 mg kg^{-1} day^{-1} for the 0–5- and 5–20-cm layers, respectively. LL-applied soils had peak CO_2 evolution rates of 31.68 (0–5-cm layer) and 17.8 (5–20-cm

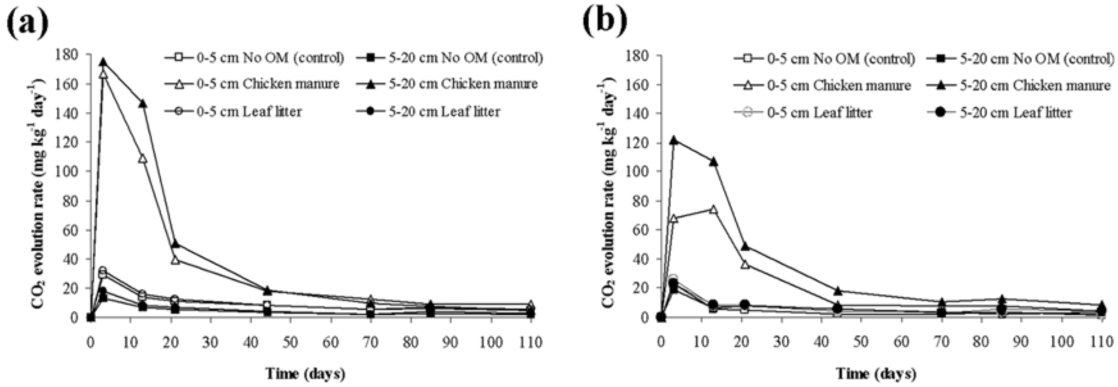

Figure 1. Carbon dioxide evolution rate (mg kg^{-1} day^{-1}) in the 0–5- and 5–20-cm depths of (a) Bagabag, Philippines and (b) Tsumagoi, Japan soils over 110 days of incubation following application of chicken manure and leaf litter.

layer) mg kg^{-1} day^{-1}. Control soils peaked at 29.44 (0–5-cm layer) and 13.14 (5–20-cm layer) mg kg^{-1} day^{-1}. High CO_2 evolution extended up to three weeks, especially in the CM-applied soils, where 63.36% and 72.14% of the cumulative CO_2 evolution was released during that period. CO_2 evolution during the same period in the LL-applied and control soils only ranged from 36.61 – 39.98% of cumulative CO_2 evolution, showing the higher capacity of chicken manure to induce higher CO_2 evolution than leaf litter.

[9] Peak CO_2 evolution in the Tsumagoi soil also occurred within 3 days after FOM addition, except in the 0–5-cm layer applied with CM, which peaked within 3–13 days. The LL-applied soils had peak CO_2 evolution rates of 25.89 (0–5-cm layer) and 23.03 (5–20-cm layer) mg kg^{-1} day^{-1}. The CM-applied soils had peak 74.55 and 121.67 mg kg^{-1} day^{-1} in the 0–5- and 5–20-cm layers, respectively. The control soils peaked 22.77 (0–5-cm layer) and 19.26 (5–20-cm layer) mg kg^{-1} day^{-1}. Average CO_2 evolution was highest in the CM-applied soils: 17.56 (0–5-cm layer) and 27.58 mg kg^{-1} day^{-1} (5–20-cm layer). The application of LL caused average CO_2 evolution of 5.07 mg kg^{-1} in the 0–5-cm layer and 5.94 mg kg^{-1} day^{-1} in the 5–20-cm layer. In the control soil, average CO_2 evolution was 3.65 mg kg^{-1} (0–5-cm) and 5.12 mg kg^{-1} (5–20-cm).

5.2. Mineral-Associated Organic Carbon

[10] The short-term changes in the mineral-associated organic carbon (MAOC) of the two soils are shown in Figure 2. In the Bagabag soil, original (zero-day level) MAOC of the surface (0–5 cm) and 5–20-cm layers were 11.53 and 10.02 g kg^{-1}, respectively (Table 2). In the control soils, MAOC decreased to 11.38 (0–5-) and 9.85 (5–20-cm layer) g kg^{-1} after 110 incubation days and corresponded to a decline of 1.3 and 1.7% of the original level. For the CM-applied soils, MAOC after 110 days decreased to 10.65 and 9.43 in the 0–5- and 5–20-cm layers, corresponding to 7.63 and 5.89% decline from the original value. In the LL-applied soils, MAOC increased in the 0–5-cm depth by 8.67% of original MAOC. In the 5–20-cm depth, it dropped to 9.73 g kg^{-1} after 110 days, a decrease of 2.89% of the initial MAOC.

[11] In Tsumagoi soil, original MAOC was lower in the 0–5-cm layer (33.93 g kg^{-1}) than in the 5–20-cm layer (38.19 g kg^{-1}). At the end of incubation, MAOC in the control soil reduced to 31.92 and 33.61 g kg^{-1} for the 0–5- and 5–20-cm layers, respectively, with a corresponding decline of 5.92 and 11.99% relative to the original level. For the CM-applied soils, MAOC increased by 0.59 and dropped by 6.36% in the 0–5- and 5–20-cm layers, respectively. The LL-applied soils also decreased by 0.47

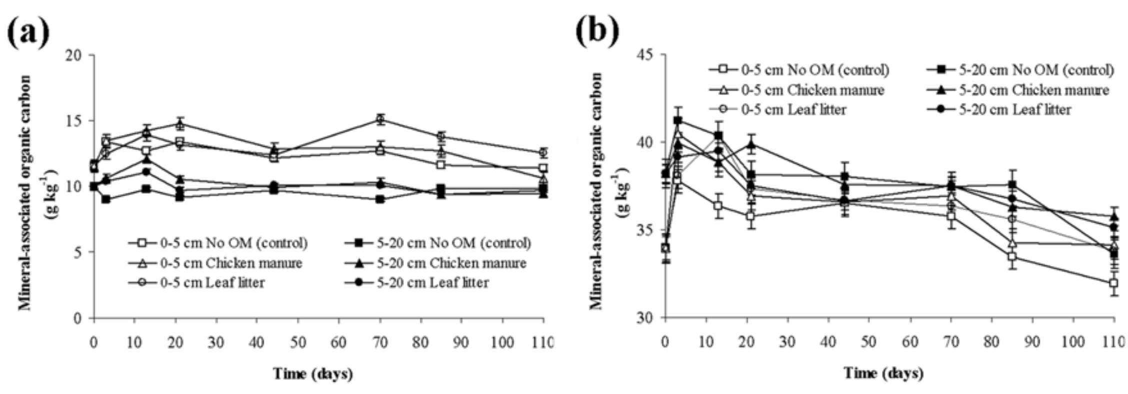

Figure 2. Change in the MAOC of the 0–5- and 5–20-cm layers of (a) Bagabag, Philippines and (b) Tsumagoi, Japan soils over 110 days of incubation following application of chicken manure and leaf litter.

Table 2. Effect of FOM × 110-Day Incubation in the MAOC Fraction of the 0–5 and 5–20 cm Depths of Two Asian Soils[a]

Depth (cm)	Days After Incubation	Treatment	MAOC[b] ($g\ kg^{-1}$) Bagabag Soil	Tsumagoi Soil
0–5	0	No OM (control)	11.53 (c)	33.93 (a, b)
	110	No OM (control)	11.38 (b, c)	31.92 (a)
	110	Leaf litter (1.81 $g\ kg^{-1}$)	12.53 (c)	33.77 (a, b)
	110	Chicken manure (2.12 $g\ kg^{-1}$)	10.65 (a, b)	34.13 (a, b)
5–20	0	No OM (control)	10.02 (a)	38.19 (c)
	110	No OM (control)	9.85 (a)	33.61 (a, b)
	110	Leaf litter (1.81 $g\ kg^{-1}$)	9.73 (a)	35.12 (b)
	110	Chicken manure (2.12 $g\ kg^{-1}$)	9.43 (a)	35.76 (b)

[a]Soils were collected from Bagabag, Nueva Vizcaya, Philippines and Tsumagoi, Gunma Prefecture, Japan.
[b]Means are significant at $p < 0.05$. Letters in parentheses indicate which means are statistically comparable.

and 8.04% in the 0–5- and 5–20-cm layers, respectively, after 110 incubation days.

6. Discussion

6.1. CO_2 Evolution

[12] Our results were consistent with *Calderon et al.* [2004] who observed that CO_2 flux peaked in manured soil during the first week after manure application. *Hamer and Marschner* [2005] observed that CO_2 flux occurred 2.4 days after substrate addition. *Fontaine et al.* [2003, 2004] showed that the release of extra carbon occurs immediately after the addition of OM. *Chotte et al.* [1998] observed highest CO_2 mineralization during the first 3 days and *Luna-Guido et al.* [2001] reported sharp increase in CO_2 "in the first days of incubation".

[13] Cumulative CO_2 evolution was significantly higher in the CM- than in the LL-applied and control in both Bagabag and Tsumagoi soils (Table 3). CO_2 evolution between the control and LL-applied soils was comparable. This trend suggests the greater ability of CM to induce CO_2 evolution than leaf litter in both soils. Aside from containing more C than leaf litter, chicken manure has a narrower C/N ratio, indicating the availability of energy that can enhance microbial respiration. Chicken manure exhibits considerable heterogeneity in terms of nutrient composition [*Lhadi et al.*, 2006; *Tiquia*, 2002; *El Nadi et al.*, 1995], diversity in microbial population [*Tiquia*, 2002; *El Nadi et al.*, 1995] that explains the availability of extracellular enzymes that may be involve in CO_2 evolution, and physico-chemical characteristics [*Sellami et al.*, 2008; *Hachicha et al.*, 2008]. *De Nobili et al.* [2001] noted that the application of "trigger solutions" caused a rapid increase in the metabolic activity of microbial biomass. Several authors stated the direct relationship between CO_2 production and microbial biomass [*Kuzyakov et al.*, 2000]. The exhaustion of the readily-available substrates from the FOMs caused CO_2 evolution to start leveling off after a brief peak 3 days after incubation in the leaf litter-applied soils. This period extended until 44 days after incubation in the chicken manure-applied soils. This seems to suggest that with the exhaustion of readily-available substrates from the FOM, continued CO_2 evolution could have been due to the mineralization of C from a different source not from the FOM.

[14] Although Tsumagoi soil had higher initial total SOC and MAOC than the Bagabag soil in the two depths, Bagabag soil evolved more CO_2 (Figure 1). One of the main reasons for the smaller CO_2 evolution of Tsumagoi soil could be due to the stabilization of humus by complexation with aluminum in volcanic ash soils [*Nanzyo*, 2002] and the presence of these Al-humus complexes gave the humus stronger stability [*Shirato et al.*, 2004] which provided further resistance to microbial degradation. According to *Nanzyo* [2002], large amounts of humus are stored in the A and buried A horizons of Andisols. Organic C content of Andisols ranges between 0 and about 200 g kg^{-1}. This implies the capacity of volcanic ash soils to retain more of input C probably due to the ability of these soils to bind humus with aluminum and form Al-humus complexes which can hold and stabilize SOM. It can be generally assumed that the more labile fraction SOC has, the greater is the soil's tendency to evolve more CO_2 in the short-term. This suggests that other soil-related factors influence CO_2 evolution from soils. One of them is the capacity of soils to bind humus to form complexes resistant to microbial degradation.

6.2. Mineral-Associated Organic Carbon

[15] The increase in MAOC in the LL-applied 0–5-cm depth of the Bagabag soil after 110 days was not significant (Table 2). On the other hand, the decrease in MAOC in the CM-applied soils was significantly lower than the initial MAOC value (zero day level). In the 5–20-cm depth, MAOC values of all treatments were comparable to the

Table 3. Effect of FOM Application on the CO_2 Evolution of 0–5 and 5–20 cm Depths of Two Asian Soils[a]

Treatment	Depth (cm)	Cumulative CO_2 Evolution[b] ($mg\ kg^{-1}$) Bagabag Soil	Tsumagoi Soil
No OM (control)	0–5	906.82 (a)	401.13 (a)
	5–20	396.96 (a)	563.34 (a)
Leaf litter (1.81 $g\ kg^{-1}$)	0–5	1000.03 (a)	558.2 (a)
	5–20	525.82 (a)	653.3 (a)
Chicken manure (2.12 $g\ kg^{-1}$)	0–5	3100.38 (c)	1931.06 (b)
	5–20	3375.04 (c)	3033.95 (c)

[a]Soils were collected from Bagabag, Nueva Vizcaya, Philippines and Tsumagoi, Gunma Prefecture, Japan.
[b]Means are significant at $p < 0.05$. Letters in parentheses indicate which means are statistically comparable.

zero-day value. This trend suggests that chicken manure application might cause greater decline in MAOC in this soil. *Fontaine et al.* [2003] proposed a mechanism that leads to extra CO_2 evolution that r-strategists microorganisms produce extra cellular enzymes which are both efficient in degrading SOM. In this scenario, FOM application could give rise to an increase in r-strategist microorganisms thereby producing more extracellular enzymes which can also degrade the SOM. This mechanism can explain the behavior of MAOC in the Bagabag soil, where higher amount of MAOC was turned over in the CM-applied soils than in the control.

[16] In the Tsumagoi soil, changes in MAOC in all treatments in the 0–5-cm layer did not significantly differ with the initial MAOC. However, in the 5–20-cm layer, the control soil did cause the biggest decline in MAOC after 110 incubation days. LL and CM application also caused significant decline from the original MAOC. This suggests significant turnover of the stable MAOC in a short-term even with FOM application in this soil. The MAOC is not always resistant to microbial attack, and may affect short-term C dynamics in soils.

[17] We observed "add and subtract" changes in the MAOC between measurement dates during the incubation period, particularly in the early stage of incubation (Figure 2). *Haile-Mariam et al.* [2008] disclosed that SOM is a continuum of materials from very young to very old with ongoing transfers between pools. This denotes that SOM moves to and from one fraction to another and associates with the particle size fractions.

[18] Findings of previous studies [*Gerzabek et al.*, 1997, 2001; *Dalenberg and Jager*, 1989] on SOC gains due to long-term C input were encouraging as these seem to prove the ability of soils to store carbon in the long-term. However, it is imperative to elucidate the recalcitrance of this MAOC-derived C that is lost in the short-term. It is highly possible that this significant MAOC-derived C turnover came from the most recalcitrant and oldest SOM fraction. This could have big impact on the overall terrestrial carbon dynamics if the most stable SOC with long turnover times are lost in exchange of the less stable SOC that moves into the fine soil fractions during carbon input to soil.

[19] **Acknowledgments.** We acknowledge the assistance of the laboratory members of Soil Physics and Soil Hydrology, Department of Biological and Environmental Engineering, The University of Tokyo, for their help in soil sampling in Japan. Special thanks also to Hiromi Imoto for his analytical and technical assistance during the experiment. We also thank Ludovico Pabio, Israel Dumale, Jun Gatchalian, and El Dumale for their assistance in soil sampling in the Philippines.

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